

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k163590

B. Purpose for Submission:

New device

C. Measurand:

Benzodiazepines

D. Type of Test:

Qualitative screening test: enzyme immunoassay (EIA)

Quantitative confirmatory test: LC/MS/MS

E. Applicant:

Psychemedics Corporation

F. Proprietary and Established Names:

Psychemedics Microplate EIA for Benzodiazepines in Hair

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
JXM	Class II	21 CFR 862.3170	Toxicology (91)

H. Intended Use:

1. Intended use(s):

Refer to Indications for Use below.

2. Indication(s) for use:

The Psychemedics Microplate EIA For Benzodiazepines in Hair is an in vitro diagnostic device for the qualitative detection of benzodiazepines in hair. The assay is intended for

use in workplace settings for the qualitative analysis of human head and body hair. The assay uses a cutoff calibrator of 1 ng oxazepam/10 mg hair.

Psychemedics plans to perform this test at one site. Psychemedics has not performed an evaluation of reproducibility at different laboratories.

The Psychemedics Microplate EIA For Benzodiazepines in Hair provides only a preliminary analytical test result. A more specific alternate chemical method must be used to obtain a confirmed analytical result. Liquid Chromatography/Mass spectrometry/Mass spectrometry (LC/MS/MS) using deuterated internal standards in multiple reaction monitoring (MRM) mode is the confirmatory method used by Psychemedics Corporation. This confirmatory method uses a cutoff of 0.2 ng of the identified benzodiazepine/10 mg hair.

3. Special conditions for use statement(s):

This assay is for over the counter use.

The Psychemedics Microplate EIA for Benzodiazepines in Hair combines a screening method (immunoassay) with a confirmation method (LC/MS/MS) in one test system.

4. Special instrument requirements:

The device is for use with a microplate reader capable of measuring at 450 and 630 nm. Plate washing also requires an instrument specifically designed to effectively and reproducibly wash all wells uniformly.

For confirmation testing, Psychemedics uses a AB Sciex API 3200 LC/MS/MS (Serial numbers AA24661109 and AA28841310), in multiple reaction monitoring (MRM) mode, linked to two Shimadzu LC-20AD Micro pumps and a Leap Technologies PAL autosampler, along with deuterated internal standards.

I. Device Description:

The screening assay consists of:

- BSA-oxazepam coated Microplate
- Cutoff Calibrator (.8 ng oxazepam/37 uL) added to 8 mg hair
- Controls (minus 50% of cutoff, and plus 100% of cutoff)
- Primary antibody against oxazepam and other benzodiazepines
- HRP-labeled secondary antibody directed against the primary antibody species
- Substrate TMB (tetramethylbenzidine)
- Acidic stop solution (2 N HCl)
- Hair sample collection kit

The confirmation assay consists of a Perkin Elmer Sciex API 3200 LC/MS/MS (Serial

numbers AA24661109 and AA28841310) linked to two Shimazu LC-20AD Micro pumps and a Leap Technologies PAL autosampler, an internal standard (0.2 ng oxazepam/10 mg hair), Signal peak identification and integration are performed with AB Sciex MultiQuant Software, version 1.5.1 and 1.6.2, using the SignalFinder Integration Algorithm that is a feature of the MultiQuant Software.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Orasure Technologies Benzodiazepines Intercept Micro-plate EIA

2. Predicate 510(k) number(s):

k013882

3. Comparison with predicate:

Similarities		
Item	Device: Psychemedics Microplate EIA for Benzodiazepines in Hair	Predicate: k013882 Orasure Benzodiazepine Intercept Micro-plate EIA
Intended Use	Same	Qualitative detection of benzodiazepines
Methodology	Same	Enzyme Immunoassay read at 450 nm
Calibrator Drug	Same	Oxazepam

Differences		
Item	Device: Psychemedics Microplate EIA for Benzodiazepines in Hair	Predicate: k013882 Orasure Benzodiazepine Intercept Micro-plate EIA
Matrix	Hair	Oral Fluid
Confirmation Method	LC/MS/MS	GC/MS/MS
Cutoff	1 ng / 10 mg hair	1 ng / mL oral fluid
Intended User	Intended to be used in house at Psychemedics only	Intended to be marketed to individual laboratories
Extraction Step	Required; performed in- house	Not required

K. Standard/Guidance Document Referenced (if applicable):

None referenced

L. Test Principle:

The Psychomedics Microplate EIA For Benzodiazepines in Hair consists of (1) a screening immunoassay and (2) a confirmation test by LC-MS/MS.

The screening immunoassay consists of two parts; a pre-analytical hair treatment procedure (to remove benzodiazepines from solid hair matrix to a measurable liquid matrix), and the qualitative assay. The qualitative assay is an Enzyme-Linked ImmunoSorbent Assay (ELISA) based upon the competitive binding of benzodiazepines in the sample and solid-phase oxazepam in the microplate wells to the assay detection antibody. Sample is added to the microplate well, followed by primary antibody (monoclonal mouse anti-oxazepam), and then (after washing the plate) goat anti-mouse-horseradish peroxidase conjugate is added. Enzyme substrate is then added and the plate is read on a microplate reader at 450 nm.

Confirmatory testing:

Confirmation testing of samples is performed on a 6-12 mg aliquot of the original hair specimen. The hair is washed with isopropanol, followed by phosphate buffer. After washing, samples are then spiked with deuterated benzodiazepines (Alprazolam-D5, Lorazepam-D4, Diazepam-D5, Nordiazepam-D5, Oxazepam-D5, and Temazepam-D5) to a concentration of 1.0 ng/10 mg hair, processed by solid phase extraction and analyzed using the Perkin Elmer Sciex API 3200 LC/MS/MS operating in the Positive Multiple Reaction Mode. To account for variability in drug recovery during solid phase extraction the recovered concentration of the deuterated internal standards in each sample is used to normalize the data for that sample.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Precision of the immunoassay screening method

Hair samples previously tested and shown to be negative for benzodiazepines were spiked with oxazepam at the following concentrations: 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, and 2 ng/10 mg hair (negative, $\pm 75\%$, $\pm 50\%$, $\pm 25\%$, cutoff, and $+100\%$ of the cutoff). Intra-Assay Precision was demonstrated by measuring these nine concentrations of oxazepam in hair samples in replicates of 15 within a single run. Results are summarized below.

Concentration relative to cutoff (1 ng/10 mg hair)	No. Samples	Negative / Positive
Negative	15	15/0
25%	15	15/0
50%	15	15/0
75%	15	15/0
Cutoff	15	7/8
125%	15	0/15
150%	15	0/15
175%	15	0/15
200%	15	0/15

Hair samples previously tested and shown to be negative for benzodiazepines were spiked with oxazepam at the following concentrations: 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, and 2 ng/10 mg hair (negative, $\pm 75\%$, $\pm 50\%$, $\pm 25\%$, cutoff, and $+100\%$ of the cutoff). Inter-Assay Precision of the assay was evaluated by measuring these nine concentrations of oxazepam in hair samples over the course of five days, in replicates of 15 per day. Results are summarized below.

Concentration relative to cutoff (1 ng/10 mg hair)	No. Samples	Positive / Negative
Negative	75	75/0
25%	75	75/0
50%	75	75/0
75%	75	75/0
Cutoff	75	43/32
125%	75	0/75
150%	75	0/75
175%	75	0/75
200%	75	0/75

Precision of the LC-MS/MS method

Intermediate precision around the LC/MS/MS cutoff was conducted using drug negative hair samples spiked with 0.05, 0.1, 0.15, 0.2, 0.25, or 0.3 ng/10 mg hair. Samples were prepared in replicates of 5 per each concentration, and run over 5 days for 25 determinations per drug. Results for the six benzodiazepines measured by the LC-MS/MS assay are summarized below.

	ALPRAZOLAM (ng/10 mg hair)					
<u>Concentration</u>	<u>0.05</u>	<u>0.10</u>	<u>0.15</u>	<u>0.20</u>	<u>0.25</u>	<u>0.30</u>
Average	0.0510	0.1031	0.1513	0.2088	0.2551	0.3128
S.D.	0.0049	0.0093	0.0100	0.0109	0.0111	0.0188
% CV	9.66	8.99	6.59	5.23	4.37	6.00
Percent of Target	102.1	103.1	100.9	104.4	102.0	104.3

	LORAZEPAM (ng/10mg hair)					
<u>Concentration</u>	<u>0.05</u>	<u>0.10</u>	<u>0.15</u>	<u>0.20</u>	<u>0.25</u>	<u>0.30</u>
Average	0.05088	0.10128	0.1502	0.19632	0.24548	0.29756
S.D.	0.0046	0.0060	0.0104	0.0144	0.0123	0.0164
% CV	9.03	5.93	6.92	7.34	5.02	5.52
Percent of Target	101.8	101.3	100.1	98.2	98.2	99.2

	DIAZEPAM (ng/10 mg hair)					
<u>Concentration</u>	<u>0.05</u>	<u>0.10</u>	<u>0.15</u>	<u>0.20</u>	<u>0.25</u>	<u>0.3</u>
Average	0.0498	0.1035	0.1587	0.2095	0.2661	0.3179
S.D.	0.0047	0.0062	0.0113	0.0143	0.0131	0.0190
% CV	9.51	6.01	7.12	6.84	4.92	5.99
Percent of Target	99.7	103.5	105.8	104.8	106.4	106.0

	NORDIAZEPAM (ng/10mg hair)					
<u>Concentration</u>	<u>0.05</u>	<u>0.10</u>	<u>0.15</u>	<u>0.20</u>	<u>0.25</u>	<u>0.30</u>
Average	0.0490	0.0990	0.1502	0.1960	0.2478	0.3044
S.D.	0.0043	0.0068	0.0103	0.0097	0.0149	0.0171
% CV	8.72	6.83	6.86	4.93	6.01	5.62
Percent of Target	98.0	99.0	100.1	98.0	99.1	101.5

	OXAZEPAM (ng/10mg hair)					
<u>Concentration</u>	<u>0.05</u>	<u>0.10</u>	<u>0.15</u>	<u>0.20</u>	<u>0.25</u>	<u>0.30</u>
Average	0.0497	0.1016	0.1554	0.1991	0.2576	0.3102
S.D.	0.0042	0.0082	0.0120	0.0105	0.0130	0.0115
% CV	8.50	8.10	7.71	5.29	5.06	3.72
Percent of Target	99.4	101.6	103.6	99.5	103.1	103.4

	TEMAZEPAM (ng/10ng hair)					
<u>Concentration</u>	<u>0.05</u>	<u>0.10</u>	<u>0.15</u>	<u>0.20</u>	<u>0.25</u>	<u>0.30</u>
Average	0.0504	0.0978	0.1495	0.1993	0.2492	0.2996
S.D.	0.0029	0.0060	0.0070	0.0065	0.0086	0.0070
% CV	5.67	6.17	4.65	3.27	3.45	2.32
Percent of Target	100.7	97.8	99.7	99.7	99.7	99.9

Within run precision throughout the LC/MS/MS range was conducted using drug negative hair samples spiked with 0.05, 0.1, 0.15, 0.2, 1.0, 10.0, 15.0, or 20.0 ng/10 mg hair. Samples were prepared in replicates of 5, per each concentration, and concentrations were determined in one run. Results for the six benzodiazepines measured by the LC-MS/MS assay are summarized below.

	Target Concentration (ng/10 mg hair)							
	0.05	0.10	0.15	0.20	1.0	10	15	20
Alprazolam concentration	0.056	0.100	0.158	0.234	0.947	10.395	15.898	20.496
	0.051	0.092	0.148	0.217	0.953	10.279	15.885	19.164
	0.054	0.093	0.162	0.222	0.903	10.430	15.934	20.332
	0.045	0.095	0.161	0.200	0.944	10.670	14.732	20.049
	0.053	0.090	0.164	0.198	0.947	10.196	15.577	20.197
Average	0.0518	0.094	0.1586	0.2142	0.9388	10.394	15.605	20.0476
S.D.	0.004	0.004	0.006	0.015	0.020	0.180	0.509	0.521
% CV	8.12	4.05	3.98	7.10	2.16	1.73	3.26	2.60
% of Target	103.6	94.0	105.7	107.1	93.9	103.9	104.0	100.2
Lorazepam concentration	0.052	0.085	0.154	0.22	0.906	10.191	15.072	19.987
	0.044	0.092	0.147	0.21	0.923	10.165	14.604	18.966
	0.05	0.092	0.148	0.204	0.895	10.262	14.41	19.077
	0.05	0.102	0.137	0.2	0.92	10.429	15.425	19.461
	0.054	0.1	0.163	0.218	0.905	10.927	14.393	18.933
Average	0.05	0.0942	0.1498	0.2104	0.9098	10.394	14.780	19.2848
S.D.	0.004	0.007	0.010	0.009	0.012	0.315	0.452	0.445
% CV	7.48	7.29	6.39	4.11	1.27	3.03	3.06	2.31
% of Target	100.0	94.2	99.9	105.2	91.0	103.9	98.5	96.4

Diazepam concentration	0.053	0.088	0.15	0.211	0.928	10.417	14.39	18.679
	0.054	0.092	0.151	0.201	0.945	10.354	14.444	18.06
	0.044	0.084	0.163	0.203	0.96	10.561	14.943	19.458
	0.049	0.085	0.143	0.196	0.949	10.03	14.692	19.613
	0.054	0.094	0.167	0.209	0.936	10.439	14.661	19.133
Average	0.0508	0.0886	0.1548	0.204	0.9436	10.360	14.626	18.9886
S.D.	0.004	0.004	0.010	0.006	0.012	0.199	0.221	0.630
% CV	8.51	4.89	6.40	2.98	1.30	1.92	1.51	3.32
% of Target	101.6	88.6	103.2	102.0	94.4	103.6	97.5	94.9

	Target Concentration (ng/10 mg hair)							
	0.05	0.10	0.15	0.20	1.0	10	15	20
Nordiazepam concentration	0.049	0.086	0.151	0.201	0.904	10.659	14.942	19.881
	0.048	0.088	0.155	0.2	0.947	10.654	14.836	19.869
	0.056	0.092	0.14	0.201	0.937	10.278	14.917	20.328
	0.047	0.093	0.161	0.213	0.933	10.738	14.94	19.692
	0.044	0.095	0.163	0.208	0.902	10.132	14.717	19.635
Average	0.0488	0.0908	0.154	0.2046	0.9246	10.492	14.870	19.881
S.D.	0.004	0.004	0.009	0.006	0.020	0.269	0.096	0.272
% CV	9.10	4.08	5.95	2.78	2.20	2.57	0.65	1.37
% of Target	97.6	90.8	102.7	102.3	92.5	104.9	99.1	99.4
Oxazepam concentration	0.058	0.101	0.168	0.21	0.926	9.841	15.053	19.451
	0.05	0.093	0.168	0.211	0.93	10.277	14.791	19.701
	0.049	0.099	0.161	0.198	0.903	10.146	14.561	19.889
	0.048	0.091	0.158	0.2	0.905	10.394	15.271	19.595
	0.055	0.093	0.162	0.198	0.878	10.417	15.164	19.593
Average	0.052	0.0954	0.1634	0.2034	0.9084	10.215	14.968	19.6458
S.D.	0.004	0.004	0.004	0.007	0.021	0.235	0.289	0.162
% CV	8.27	4.54	2.72	3.22	2.30	2.30	1.93	0.83
% of Target	104.0	95.4	108.9	101.7	90.8	102.2	99.8	98.2

Temazepam concentration	0.049	0.089	0.149	0.201	0.907	9.51	14.172	17.948
	0.053	0.089	0.148	0.193	0.87	9.741	13.697	17.618
	0.048	0.087	0.153	0.196	0.888	9.631	14.041	17.822
	0.051	0.1	0.148	0.202	0.91	9.642	13.891	18.113
	0.045	0.105	0.159	0.19	0.868	9.641	13.755	17.754
Average	0.0492	0.094	0.1514	0.1964	0.8886	9.633	13.911	17.851
S.D.	0.003	0.008	0.005	0.005	0.020	0.082	0.197	0.189
% CV	6.16	8.51	3.12	2.61	2.23	0.85	1.42	1.06
% of Target	98.4	94.0	100.9	98.2	88.9	96.3	92.7	89.3

A second study was conducted for precision using triplicate analyses of authentic (non-spiked) benzodiazepine-positive samples. All six benzodiazepines measured with the confirmation method were included in the study. Each replicate measurement was made from a separate hair sample that was taken through the entire extraction process prior to analysis. Results for the six benzodiazepines measured by the LC-MS/MS assay are summarized below.

Alprazolam (ng/10 mg hair)					
Sample #	1	2	3	4	5
Replicate 1	6.477	0.199	2.941	7.408	3.802
Replicate 2	6.713	0.202	2.886	7.702	4.746
Replicate 3	5.711	0.243	3.159	7.269	4.189
Mean	6.3	0.2	3.0	7.5	4.2
Standard Deviation	0.5	0.0	0.1	0.2	0.5
%CV	8.3	11.5	4.8	3.0	11.2

Lorazepam (ng/10 mg hair)				
Sample #	1	2	3	4
Replicate 1	1.174	0.555	0.536	0.341
Replicate 2	1.222	0.643	0.57	0.375
Replicate 3	1.102	0.591	0.59	0.333
Mean	1.2	0.6	0.6	0.3
Standard Deviation	0.1	0.0	0.0	0.0
%CV	5.2	7.4	4.8	6.4

Diazepam (ng/10 mg hair)					
Sample #	1	2	3	4	5
Replicate 1	0.229	8.365	1.417	4.642	0.77
Replicate 2	0.235	8.443	1.572	3.75	0.903
Replicate 3	0.205	8.519	1.215	3.929	0.818
Mean	0.2	8.4	1.4	4.1	0.8
Standard Deviation	0.0	0.1	0.2	0.5	0.1
%CV	7.1	0.9	12.8	11.5	8.1

Nordiazepam (ng/10 mg hair)				
Sample #	1	2	3	4
Replicate 1	9.227	3.377	0.884	0.25
Replicate 2	9.928	3.725	0.923	0.226
Replicate 3	10.258	3.202	0.954	0.252
Mean	9.8	3.4	0.9	0.2
Standard Deviation	0.5	0.3	0.0	0.0
%CV	5.4	7.8	3.8	6.0

Oxazepam (ng/10 mg hair)				
Sample #	1	2	3	4
Replicate 1	0.176	0.262	0.161	0.073
Replicate 2	0.179	0.267	0.149	0.071
Replicate 3	0.146	0.274	0.143	0.066
Mean	0.2	0.3	0.2	0.1
Standard Deviation	0.0	0.0	0.0	0.0
%CV	10.9	2.3	6.1	5.2

Temazepam (ng/10 mg hair)					
Sample #	1	2	3	4	5
Replicate 1	1.342	0.22	0.141	0.133	0.136
Replicate 2	1.327	0.22	0.147	0.129	0.139
Replicate 3	1.291	0.233	0.114	0.136	0.132
Mean	1.3	0.2	0.1	0.1	0.1
Standard Deviation	0.0	0.0	0.0	0.0	0.0
%CV	2.0	3.3	13.1	2.6	2.6

b. Linearity/assay reportable range:

The screening immunoassay is a qualitative test only; therefore, a linearity evaluation is not applicable.

Linearity of the LC-MS/MS confirmation method

The linearity of the confirmation method was evaluated by spiking each of the benzodiazepine analytes (Alprazolam, Lorazepam, Diazepam, Nordiazepam, Oxazepam, and Temazepam) into a negative hair over the desired linear range of 0.05 to 20.0 ng/10 mg hair. Eight concentrations within this range plus a zero concentration sample were evaluated (0, 0.05, 0.1, 0.15, 0.2, 1.0, 10.0, 15.0, or 20.0 ng/10 mg hair). All levels for all benzodiazepines demonstrated percent recoveries within +/- 10% of the expected value.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Controls and calibrators are prepared as a methanolic solution containing oxazepam, and are traceable to a commercial oxazepam source. The value of the cutoff for oxazepam is 1 ng/10 mg hair.

Stability studies for both controls and calibrators have been conducted. Protocols and acceptance criteria were described and found to be acceptable. The manufacturer claims the following expiration date for both controls and calibrators: When stored at less than or equal to -10° C the calibrators and controls are stable for one year.

Shipping stability: 19 hair samples containing benzodiazepines were shipped overnight to another location, held for four days, and then returned to Psychemedics by overnight shipping. Samples were tested by the routine LC/MS/MS procedure both before being shipped and after returning to Psychemedics. All six benzodiazepines were evaluated, and recoveries after shipping were found to be acceptable.

Sample storage stability: 21 hair samples were stored in the same collection foil and card-envelope provided with the hair collection kit, following the instructions provided in the package insert. Samples were tested by LC/MS/MS on day 0, and again at 12 months, following storage at 20° – 24° C (68° – 75° F). All six benzodiazepines were evaluated, and recoveries after storage were found to be acceptable.

d. Detection limit:

The screening immunoassay is a qualitative test only; therefore, a detection limit evaluation is not applicable. See section M.1.a. above for sensitivity around the immunoassay device cutoff.

Detection Limit of the LC-MS/MS method

The sponsor performed a study to determine the Lower limit of Quantitation (LLOQ) of the LC-MS/MS assay for each of the six benzodiazepines in the assay. Four types of hair (Caucasian, Hispanic, African-American, and Asian) were obtained and were cut into three segments for a total of 12 hair segments to be analyzed per drug. Each of the 12 hair segments was independently spiked with each specific benzodiazepine and analyzed according to the LC-MS/MS procedure stated in the package insert.

The results from the study support an LLOQ of 0.05 ng/10 mg hair, and a reportable range of 0.05 to 20.0 ng/10 mg hair for all six drugs.

e. *Analytical specificity:*

Specificity of the immunoassay screening method

Immunoassay cross-reactivity with structurally related compounds

The cross-reactivity characteristics of the screening immunoassay was evaluated by spiking various concentrations of benzodiazepines into drug-free hair samples and comparing the result to the cutoff calibrator. The table below lists the percent cross-reactivity and the approximate concentration of each compound required to produce a response approximately equivalent to the cutoff concentration of the assay.

Compound	Percent Cross-reactivity	Expected Concentration in ng/10 mg hair equivalent to cutoff calibrator
Clobazam	550	0.18
Diazepam	550	0.18
Nimetazepam	550	0.18
Estazolam	400	0.25
Temazepam	312	0.32
Alprazolam	277	0.36
Flunitrazepam	222	0.45
Nordiazepam	220	0.45
Nitrazepam	220	0.45
N-Desmethyflunitrazepam	172	0.58
Lormetazepam	133	0.75
Oxazepam	100	1
Midazolam	42	2.4

Compound	Percent Cross-reactivity	Expected Concentration in ng/10 mg hair equivalent to cutoff calibrator
Clonazepam	33	3
Triazolam	31	3.2
alpha-Hydroxyprazolam	22	4.5
Lorazepam	13	7.5
Bromazepam	8.3	12
Chlordiazepoxide	4	25
7-Aminoflunitrazepam	3.1	32
Hydroxymethylflurazepam	2	50
Alpha-Hydroxytriazolam	1.7	60
desalkylflurazepam	1	100
7-Aminonitrazepam	0.6	170
7-aminoclonazepam	<0.3	>300
Flurazepam	<0.3	>300
Prazepam	<0.3	>300
Zolpidem Tartrate	<0.3	>300

Interference from structurally unrelated compounds

The following potentially interfering compounds were tested using the screening immunoassay on samples spiked with oxazepam at the cutoff and at $\pm 50\%$ of the cutoff to assess possible positive or negative interference. All potential interferents listed below were tested at a concentration of 100 ng/10 mg hair. No positive or negative interference was observed in this study.

11-nor-9-Carboxy-delta-9-THC	Dextromethorphan	Naproxen
1S, 2R Ephedrine	Doxepin	Nicotine
5-hydroxyindole-3-acetic acid	Doxylamine succinate	Normetanephine (+/-)
8(-)-11-nor-9-Carboxy-delta-9 THC	Ecgononine	Nortriptyline
Acetaminophen	Erythromycin	O-Desmethyvenlafaxine
Amitriptyline	Ethosuximide	Oxycodone
Amobarbital	Ethylmorphine	Penicillin G
Amoxicillin	Fentanyl	Pentazocine
Anhydroecgonine methyl ester	Haloperidol	Phendimetrazine

Atropine	Hexobarbital	Phendimetrazine bitartrate
Benzocaine	Hydrocodone	Phenmetrazine
Benzocaine	Hydromorphone	Phenobarbital
Buprenorphine	Ibuprofen	Procaine
Bupropion	Imipramine	Promethazine
Butabarbital	Lidocaine	Propanolol
Caffeine	LSD	Propoxyphene
Cannabinol	Meperidine	Protriptyline
Chlorpheniramine maleate	Mepivacaine	R-(-)-Phenylephrine
Cis-tramadol HCl	Metanephine (+/-)	R,R(-)-Pseudoephedrine
Codeine	Methadone (+/-)	Secobarbital
Cotinine	Methanol	Streptomycin
Synthetic cannabinoids CP 47, 497 (+/-)	Methaqualone	Synthetic cannabinoid AM2201
Delta 8-THC	Methyl phenidate	Synthetic cannabinoid HU-211
Desipramine	Morphine	Synthetic cannabinoids JWH-019, 081, 122, 200, and 250
Desmethyldoxepin	Nalorphine	Thioridazine
Despiramine	Naloxone	Trimipramine
Despropionyl fentanyl	Naltrexone	Vanilmandelic acid(+/-)
		Venlafaxine hydrochloride

Effect of cosmetic treatments

To evaluate any effects of cosmetic treatments, 20 hair samples negative for benzodiazepines and 8 hair samples positive for benzodiazepines were treated with the following cosmetic treatments: permanent wave, relaxer, bleach, dye, and shampoo. After the treatments these samples and an aliquot of the same hair samples, untreated, were digested and assayed by the immunoassay screening method. All negative results remained negative and all positive results remained positive after the treatments.

The effect of cosmetic treatments was also evaluated using the LC-MS/MS method, for the same samples. The sponsor determined the concentrations of drug before and after treatment (% recovery in parentheses) and the results are provided below (ND = not detected in either untreated or treated samples).

Effect of Dye Product #2 on Benzodiazepines in Hair – ng/10 mg hair						
Alprazolam	Lorazepam	Diazepam	Nordiazepam	Oxazepam	Temazepam	Sample #
Untreated						
ND	1.158	3.447	1.090	ND	ND	1
1.563	0.792	ND	ND	ND	ND	2
0.910	ND	ND	ND	ND	ND	3
0.992	ND	ND	ND	ND	ND	4
Dyed						
ND	0.941 (81%)	2.075 (60%)	1.195 (110%)	ND	ND	1
.901(58%)	0.740 (93%)	ND	ND	ND	ND	2
1.258(133%)	ND	ND	ND	ND	ND	3
.623(63%)	ND	ND	ND	ND	ND	4

Effect of Bleach Product #1 on Benzodiazepines in Hair – ng/10 mg hair						
Alprazolam	Lorazepam	Diazepam	Nordiazepam	Oxazepam	Temazepam	Sample #
Untreated						
ND	ND	ND	ND	ND	0.847	1
ND	ND	ND	0.613	ND	ND	2
1.737	ND	0.403 (94%)	1.128	ND	ND	3
ND	ND	3.826	5.057	ND	ND	4
Bleached						
ND	ND	ND	ND	ND	0.996 (118%)	1
ND	ND	ND	0.476 (78%)	ND	ND	2
1.500 (86%)	ND	0.378 (94%)	1.326 (118%)	ND	ND	3
ND	ND	2.930 (77%)	4.526 (89%)	ND	ND	4

Effect of Bleach Product #2 on Benzodiazepines in Hair – ng/10 mg hair						
Alprazolam	Lorazepam	Diazepam	Nordiazepam	Oxazepam	Temazepam	Sample #
Untreated						
ND	ND	1.947	5.643	0.610	0.392	1
0.876	ND	ND	ND	ND	ND	2
1.410	0.567	ND	ND	ND	ND	3
0.992	ND	ND	ND	ND	ND	4
Bleached						
ND	ND	2.25 (116%)	5.941 (105%)	0.92 (151%)	0.46 (118%)	1
0.819 (93%)	ND	ND	ND	ND	ND	2
1.330 (94%)	0.50 (88%)	ND	ND	ND	ND	3
.788 (79%)	ND	ND	ND	ND	ND	4

Effect of Relaxer Product #1 on Benzodiazepines in Hair – ng/10 mg hair						
Alprazolam	Lorazepam	Diazepam	Nordiazepam	Oxazepam	Temazepam	Sample #
Untreated						
ND	ND	ND	ND	ND	0.847	1
ND	ND	ND	0.613	ND	ND	2
ND	ND	1.101	1.702	ND	ND	3
0.226	1.158	3.447	1.070	ND	ND	4
Treated						
ND	ND	ND	ND	ND	0.985 (116%)	1
ND	ND	ND	0.641 (105%)	ND	ND	2
ND	ND	1.11 (101%)	2.051 (121%)	ND	ND	3
0.314 (139%)	1.37 (119%)	2.113 (61%)	0.970 (91%)	ND	ND	4

Effect of Relaxer Product #2 on Benzodiazepines in Hair – ng/10 mg hair						
Alprazolam	Lorazepam	Diazepam	Nordiazepam	Oxazepam	Temazepam	Sample #
Untreated						
1.369	ND	ND	ND	ND	ND	1
5.957	ND	.426	.210	ND	.496	2
1.027	ND	ND	ND	ND	ND	3
ND	ND	0.548	1.020	ND	ND	4
Relaxed						
1.228 (90%)	ND	ND	ND	ND	ND	1
3.821 (64%)	ND	.392 (92%)	.163 (78%)	ND	.319 (64%)	2
1.05 (103%)	ND	ND	ND	ND	ND	3
ND	ND	0.60 (109%)	1.172 (115%)	ND	ND	4

Effect of Shampoo Product #1 on Benzodiazepines in Hair - ng/10 mg hair						
Alprazolam	Lorazepam	Diazepam	Nordiazepam	Oxazepam	Temazepam	Sample #
Untreated						
ND	ND	ND	ND	ND	0.601	1
ND	ND	ND	6.928	ND	ND	2
ND	ND	ND	0.281	ND	ND	3
ND	ND	1.947	5.643	0.610	0.392	4
Shampoo						
ND	ND	ND	ND	ND	0.384 (64%)	1
ND	ND	ND	8.245 (119%)	ND	ND	2
ND	ND	ND	0.285 (101%)	ND	ND	3
ND	ND	2.258 (116%)	5.274 (93%)	0.77 (127%)	0.50 (128%)	4

Effect of Shampoo Product #2 on Benzodiazepines in Hair - ng/10 mg hair						
Alprazolam	Lorazepam	Diazepam	Nordiazepam	Oxazepam	Temazepam	Sample #
Untreated						
1.563	0.792	ND	ND	ND	ND	1
1.410	0.567	ND	ND	ND	ND	2
0.992	ND	ND	ND	ND	ND	3
ND	ND	0.766	1.824	ND	ND	4
Shampoo						
1.100 (70%)	0.605 (76%)	ND	ND	ND	ND	1
1.156 (82%)	0.57 (101%)	ND	ND	ND	ND	2
1.018 (103%)	ND	ND	ND	ND	ND	3
ND	ND	0.613 (80%)	1.493 (82%)	ND	ND	4

Effect of Perm Product #1 on Benzodiazepines in Hair - ng/10 mg hair						
Alprazolam	Lorazepam	Diazepam	Nordiazepam	Oxazepam	Temazepam	Sample #
Untreated						
ND	ND	ND	ND	ND	0.601	1
ND	ND	ND	ND	ND	0.847	2
ND	ND	3.826	5.057	ND	0.120	3
1.800	ND	1.241	1.214	ND	ND	4
Permed						
ND	ND	ND	ND	ND	0.274 (46%)	1
ND	ND	ND	ND	ND	0.942 (111%)	2
ND	ND	2.016 (53%)	3.177 (63%)	ND	ND	3
2.119 (118%)	ND	1.000 (81%)	1.182(97%)	ND	ND	4

Effect of Perm Product #2 on Benzodiazepines in Hair - ng/10 mg hair						
Alprazolam	Lorazepam	Diazepam	Nordiazepam	Oxazepam	Temazepam	Sample #
Untreated						
1.563	0.792	ND	ND	ND	ND	1
2.634	ND	ND	ND	ND	ND	2
5.957	ND	0.426	ND	ND	0.496	3

0.992	ND	ND	ND	ND	ND	4
Permed						
1.138 (73%)	0.575 (73%)	ND	ND	ND	ND	1
2.255 (86%)	ND	ND	ND	ND	ND	2
3.014 (51%)	ND	0.247 (58%)	ND	ND	0.276 (56%)	3
0.913 (92%)	ND	ND	ND	ND	ND	4

Specificity of the LC-MS/MS method

Cross-reactivity with structurally related compounds

The cross-reactivity characteristics of the LC-MS/MS confirmation assay were evaluated by measuring hair samples spiked with benzodiazepines at the cutoff of 0.20 ng/10 mg/hair, and also containing the following structurally related compounds at a concentration of 0.20 ng/10 mg/hair: Clonazepam, Estazolam, Flurazepam, Meprobromate, Midazolam, Nitrazepam, Prazepam, Triazolam, and Zolpidem. All of the samples spiked with the potential cross-reactant and one of the target drugs at the cutoff produced a concentration within $\pm 15\%$ of the spiked value of 0.20 ng/10 mg/hair. The sponsor concluded that these nine benzodiazepine compounds did not cross-react or cause interference with the LC-MS/MS assay.

Interference from structurally unrelated compounds

The specificity of the LC-MS/MS confirmation assay with structurally unrelated compounds was evaluated by measuring samples spiked with benzodiazepines at the cutoff of 0.20 ng/10 mg/hair, containing the following compounds at the concentrations listed: Cotinine (500 ng/10 mg hair), Nicotine (500 ng/10 mg hair), Caffeine (500 ng/10 mg hair), Ibuprofen (50 ng/10 mg hair), Naproxen (50 ng/10 mg hair), Phentermine (20 ng/10 mg hair), Pseudoephedrine (20 ng/10 mg hair), Morphine (200 ng/10 mg hair), Hydrocodone (200 ng/10 mg hair), Oxycodone (200 ng/10 mg hair), Codeine (200 ng/10 mg hair), Cocaine (200 ng/10 mg hair), Phencyclidine (200 ng/10 mg hair), Methamphetamine (200 ng/10 mg hair), Amphetamine ((200 ng/10 mg hair), Methadone (200 ng/10 mg hair), Phenobarbital (200 ng/10 mg hair), Phenytoin (200 ng/10 mg hair), Phenylephrine(200 ng/10 mg hair) , Carbamazepine (200 ng/10 mg hair), Gabapentin (500 ng/10 mg hair), Salicylic Acid (200 ng/10 mg hair), Valproic Acid (200 ng/10 mg hair), Oxcarbazepine (200 ng/10 mg hair), Propoxyphene (200 ng/10 mg hair), Acetaminophen (200 ng/10 mg hair), Norfloxacin (0.2 ng/10 mg hair), Psilocybin (200 ng/10 mg hair), Nimesulide (200 ng/10 mg hair), Etifoxine (200 ng/10 mg hair) , Chlorcyclizine (200 ng/10 mg hair), and Teriflunomide (200 ng/10 mg hair).

All of the samples spiked with the potential interfering compounds and one of the target drugs at the cutoff produced a concentration within $\pm 15\%$ of the spiked value of 0.20 ng/10 mg/hair. The sponsor concluded that the potentially interfering compounds listed above did not cross-react or cause interference with the LC-MS/MS assay.

Studies to evaluate environmental contamination

Studies were performed to evaluate environmental contamination. Two different aliquots of 10 different hair specimens, previously shown to be negative for benzodiazepines, were soaked in either a water-based solution containing 5 ng benzodiazepines /mL or a saline-based solution containing 5 ng benzodiazepines /mL, resulting in a range of benzodiazepines on the hair from below the Limit of Quantitation (LOQ) to 0.452 ng of benzodiazepines /10 mg hair, prior to washing. After washing hair samples according to the instructions in the product insert, no samples contained an amount of drug above the LOQ of the confirmatory method.

f. Assay cut-off:

Analytical performance of the device around the claimed cutoff is described in precision section M.1.a. above.

2. Comparison studies:

a. Method comparison with predicate device:

Accuracy of the immunoassay screening method

Method comparison studies were performed by comparing the results from 382 hair samples on the immunoassay screening method to the confirmatory LC/MS/MS method. The 382 samples included 318 head hair samples and 64 body hair samples.

The color of the hair samples included 159 black samples, 197 brown (from light brown to dark brown), 8 blond, 4 red, and 14 gray. The ethnicities represented included 175 Caucasian subjects, 40 African-American subjects, 125 Hispanic subjects, and 41 Asian subjects.

Immunoassay Screening Test Result	Negative by the predicate device or less than half the cutoff concentration by LC/MS/MS analysis (<0.500 ng oxazepam equivalents/10 mg hair)	Near Cutoff Negative (Between 50% below the cutoff and the cutoff concentration) ($0.500 - 1.000$ ng oxazepam equivalents/10 mg hair)	Near Cutoff Positive (Between the cutoff and 50% above the cutoff concentration) ($1.001 - 1.500$ ng oxazepam equivalents/10 mg hair)	High Positive (greater than 50% above the cutoff concentration) (>1.500 ng oxazepam equivalents/10 mg hair)
Positive	5*	19**	17	107
Negative	233	1	0	0

Discordant Results

Immunoassay Result	LC/MS/MS Result	Sum of concentrations based on cross-reactivities
POS*	0.092 ng Alprazolam	0.255
POS*	0.093 ng Alprazolam	0.258
POS*	0.196 ng Nordiazepam	0.431
POS*	0.156 ng Alprazolam	0.432
POS*	0.111 ng Nordiazepam, 0.217 ng Oxazepam	0.461
POS**	0.191 ng Alprazolam	0.529
POS**	0.201 ng Alprazolam	0.557
POS**	0.493 ng Lorazepam	0.584
POS**	0.24 ng Alprazolam	0.665
POS**	0.121 ng Diazepam	0.666
POS**	0.073 ng Diazepam, 0.133 ng Nordiazepam	0.694
POS**	0.266 ng Alprazolam	0.737
POS**	0.267 ng Alprazolam	0.74
POS**	0.267 ng Alprazolam	0.74
POS**	0.272 ng Alprazolam	0.753
POS**	0.347 ng Nordiazepam	0.763
POS**	0.283 ng Alprazolam	0.784
POS**	0.293 ng Nordiazepam, 0.158 ng Oxazepam	0.803

Immunoassay Result	LC/MS/MS Result	Sum of concentrations based on cross-reactivities
POS**	0.099 ng Diazepam, 0.131 ng Nordiazepam	0.833
POS**	0.069 ng Alprazolam, 0.303 ng Nordiazepam	0.858
POS**	0.266 ng Alprazolam, 0.023 ng Diazepam	0.863
POS**	0.322 ng Alprazolam	0.892
POS**	0.427 ng Nordiazepam	0.939
POS**	0.308 ng Temazepam	0.961

The accuracy of the LC-MS/MS method is supported by the data collected to evaluate intra-assay precision around the cutoff (see section M.1.a.). The average recovery for all concentrations for all six benzodiazepines was within $\pm 7\%$ of the target value.

Recovery study:

The sponsor conducted a study to evaluate recovery for all six benzodiazepines at concentrations of 0.2, 10, and 20 ng/10 mg hair. For each combination of drug and concentration level, nine individual hair samples were prepared and analyzed.

Results are summarized below.

Sample #	Alprazolam		Lorazepam		Diazepam	
	ng/10 mg hair	% of target	ng/10 mg hair	% of target	ng/10 mg hair	% of target
1	0.171	85.5	0.172	86	0.171	85.5
2	0.180	90.0	0.206	103	0.215	107.5
3	0.179	89.5	0.202	101	0.203	101.5
4	0.183	91.5	0.192	96	0.182	91.0
5	0.187	93.5	0.181	90.5	0.190	95.0
6	0.172	86.0	0.179	89.5	0.196	98.0
7	0.179	89.5	0.213	106.5	0.172	86.0
8	0.199	99.5	0.177	88.5	0.190	95.0
9	0.189	94.5	0.209	104.5	0.211	105.5
Average	0.182	91.1	0.192	96.2	0.192	96.1
S.D.	0.009	4.35	0.016	7.79	0.016	7.86
%CV	4.78	4.78	8.10	8.10	8.18	8.18

Sample #	Nordiazepam		Oxazepam		Temazepam	
	ng/10 mg hair	% of target	ng/10 mg hair	% of target	ng/10 mg hair	% of target
1	0.198	99	0.179	89.5	0.172	86.0
2	0.207	103.5	0.227	113.5	0.185	92.5
3	0.229	114.5	0.22	110.0	0.183	91.5
4	0.193	96.5	0.216	108.0	0.186	93.0
5	0.192	96	0.186	93.0	0.183	91.5
6	0.177	88.5	0.2	100.0	0.172	86.0
7	0.196	98	0.228	114.0	0.172	86.0
8	0.214	107	0.225	112.5	0.173	86.0
9	0.172	86	0.221	110.5	0.184	92.0
Average	0.198	98.8	0.211	105.7	0.179	89.4
S.D.	0.018	8.80	0.018	9.21	0.006	3.25
%CV	8.91	8.91	8.72	8.72	3.56	3.63

Sample #	Alprazolam		Lorazepam		Diazepam	
	ng/10 mg hair	% of target	ng/10 mg hair	% of target	ng/10 mg hair	% of target
10	9.19	91.9	9.67	96.7	10.35	103.4
11	8.91	89.1	8.68	86.8	9.11	91.1
12	9.02	90.2	8.82	88.2	10.17	101.7
13	8.92	89.2	9.50	95.0	9.14	91.4
14	8.93	89.3	9.83	98.3	9.72	97.2
15	10.51	105.1	8.68	86.8	9.23	92.3
16	9.30	93.0	8.87	88.7	10.00	100.0
17	9.84	98.4	8.75	87.5	9.79	97.9
18	9.25	92.5	9.40	94.0	9.41	94.1
Average	9.320	93.2	9.133	91.3	9.657	96.6
S.D.	0.535	5.34	0.463	4.63	0.459	4.58
%CV	5.74	5.73	5.07	5.07	4.75	4.74

Sample #	Nordiazepam		Oxazepam		Temazepam	
	ng/10 mg hair	% of target	ng/10 mg hair	% of target	ng/10 mg hair	% of target
10	10.79	107.87	10.92	109.2	9.47	94.7
11	9.91	99.13	10.10	101.0	8.54	85.4
12	9.56	95.6	10.66	106.6	8.52	85.2
13	9.68	96.8	10.34	103.4	9.05	90.5
14	9.15	91.52	9.60	96.0	8.62	86.2
15	9.47	94.7	10.27	102.7	9.34	93.4
16	10.20	101.97	10.42	104.2	9.04	90.4
17	9.57	95.72	10.21	102.1	8.63	86.3
18	9.89	98.94	8.52	85.2	8.95	89.5
Average	9.803	98.0	10.115	101.1	8.905	89.1
S.D.	0.475	4.75	0.702	7.02	0.351	3.51
%CV	4.85	4.85	6.94	6.94	3.94	3.94

Sample #	Alprazolam		Lorazepam		Diazepam	
	ng/10 mg hair	% of target	ng/10 mg hair	% of target	ng/10 mg hair	% of target
19	20.07	100.3	18.58	92.9	17.45	87.2
20	20.87	104.4	20.48	102.4	17.39	87.0
21	19.39	97.0	19.63	98.1	17.97	89.8
22	19.57	97.8	18.51	92.6	17.31	86.6
23	20.11	100.5	19.83	99.1	18.19	90.9
24	19.93	99.7	19.26	96.3	18.81	94.1
25	20.93	104.7	19.40	97.0	18.00	90.0
26	19.57	97.9	20.40	102.0	17.69	88.4
27	21.02	105.1	19.63	98.1	17.18	85.9
Average	20.16	100.8	19.52	97.6	17.78	88.9
S.D.	0.631	3.16	0.689	3.44	0.520	2.60
%CV	3.13	3.13	3.53	3.53	2.92	2.92

Sample #	Nordiazepam		Oxazepam		Temazepam	
	ng/10 mg hair	% of target	ng/10 mg hair	% of target	ng/10 mg hair	% of target
19	19.54	97.7	21.63	108.2	19.78	98.9
20	18.23	91.1	19.48	97.4	19.49	97.5
21	19.50	97.5	19.57	97.9	21.13	105.7
22	19.65	98.2	20.43	102.1	19.05	95.2
23	19.11	95.6	20.24	101.2	20.58	102.9
24	18.67	93.4	19.63	98.2	19.57	97.9
25	19.98	99.9	20.98	104.9	20.28	101.4
26	19.40	97.0	18.78	93.9	19.57	97.8
27	18.37	91.8	19.87	99.4	18.77	93.9
Average	19.16	95.8	20.07	100.3	19.80	99.0
S.D.	0.610	3.05	0.860	4.30	0.745	3.72
%CV	3.18	3.18	4.29	4.29	3.76	3.76

b. Matrix comparison:

Not applicable. The assay is intended for only one sample matrix.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

Not applicable.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.